



new product

GATEWAY™ Destination Vectors

Exhibit C

Powerful expression with seven new GATEWAY™-compatible vectors



The GATEWAY™ technology simplifies comprehensive gene analysis and protein expression. Seven new GATEWAY™ Destination vectors, including five based on the popular pcDNA, pBAD, and pYES vectors, increase your expression options for added flexibility.

GATEWAY™ simplifies functional analysis

Thorough gene analysis often requires expressing a gene in multiple systems. This is usually a tedious task, requiring numerous cloning and subcloning steps for each expression construct to be used. The GATEWAY™ technology simplifies gene analysis by allowing you to analyze your gene in multiple expression systems with just one cloning step. You'll save time and simplify your functional gene analysis research.

How GATEWAY™ works

Using GATEWAY™ is easy. First, clone your gene of interest into an entry or donor vector and then move it into various Destination, or expression, vectors for further analysis via a simple recombination reaction (figure 1). This enables quick analysis of gene expression in *E. coli*, yeast, insect, and mammalian systems without restriction enzymes, ligase,

or traditional subcloning steps. The GATEWAY™ Technology does away with time-consuming, conventional cloning methods and provides you with a simple, rapid method for thorough analysis of your gene.

figure 2 - the pcDNA-DEST47 and pcDNA-DEST53 vectors

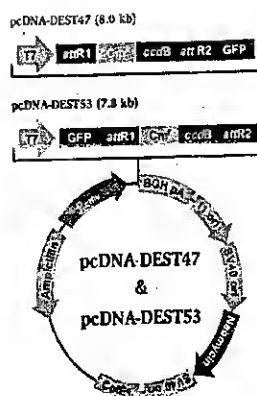
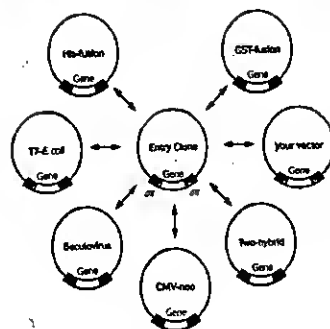


figure 1 - the power of GATEWAY™ Cloning Technology



more vectors for expression analysis

The GATEWAY™ family of vectors is always growing. To expand your options, seven of Invitrogen's most popular gene expression vectors are now GATEWAY™ compatible (table 1, page 11). pcDNA-DEST40 allows high-level expression in mammalian cells with convenient features for detection and purification of your expressed protein. The GFP fusion vectors, pcDNA-DEST47 and pcDNA-DEST53, express your protein fused to the green fluorescent protein (GFP) at the C- or

N-terminus, respectively, for non-invasive protein detection in a mammalian host (figure 2). The pEF-DEST51 vector features enhancer/promoter elements from the human elongation factor (EF-1α) subunit for high-level expression in mammalian cells. pMT-DEST48 is designed for inducible expression in the *Drosophila* Expression System (DES®). The pYES-DEST52 offers tightly regulated, high-level expression in *S. cerevisiae*. And the pBAD-DEST49 vector is specifically designed for regulated expression of thioredoxin fusion proteins in *E. coli*. Thioredoxin offers increased solubility of difficult to express proteins, improving the yield of protein production. Whichever vector you choose, you'll get powerful expression and GATEWAY™ compatibility.

figure 3 - expression of CAT/GFP in COS cells



Four micrograms of GATEWAY™-recombined pcDNA-DEST47/CAT was purified and transfected into 1×10^6 COS cells using 10 μ l of *LIPOFECTAMINE*™ 2000 transfection reagent following the standard protocol. Twenty-four hours post-transfection, the cells were analyzed using a fluorescence inverted microscope equipped with a FITC filter.

powerful expression results
To demonstrate powerful expression results with the new GATEWAY™ Destination Vectors, the CAT gene was cloned into an Entry

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GATEWAY™ Technology

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table 1 - seven new GATEWAY™ Destination Vectors

Host	Destination Vector	Parent Vector†	Benefits
Mammalian	pcDNA-DEST40	pcDNA3.1+/V5-His	<ul style="list-style-type: none"> • Full-length CMV promoter for high-level expression • C-terminal V5 epitope and 6xHis tag for convenient detection and purification of your fusion proteins
	pcDNA-DEST53	pcDNA3.1+/NT-GFP	<ul style="list-style-type: none"> • N-terminal GFP fusion for non-invasive, <i>in vivo</i> detection • Full-length CMV promoter for high-level expression
	pcDNA-DEST47	pcDNA3.1+/CT-GFP	<ul style="list-style-type: none"> • C-terminal GFP fusion for non-invasive, <i>in vivo</i> detection • Full-length CMV promoter for high-level expression
	pEF-DEST51	pEF1/V5-His	<ul style="list-style-type: none"> • Enhancer/promoter elements from the human elongation factor 1α (EF-1α) subunit for high-level expression in mammalian cells • C-terminal V5-His tag for convenient protein detection and purification
<i>Drosophila</i> S2 cells	pMT-DEST48	pMT/V5-His	<ul style="list-style-type: none"> • <i>Drosophila</i> metallothionein (MT) promoter for inducible expression in S2 cells • C-terminal V5 epitope and 6xHis tag for convenient detection and purification of your fusion proteins
Yeast	pYES-DEST52	pYES2/CT	<ul style="list-style-type: none"> • Promoter and enhancer sequences from the <i>GAL1</i> gene for tightly regulated, high-level expression in <i>S. cerevisiae</i> • C-terminal V5-His tag for convenient protein detection and purification
<i>E. coli</i>	pBAD-DEST49	pBAD/Thio-TOPO*	<ul style="list-style-type: none"> • <i>araBAD</i> promoter for tightly regulated expression in <i>E. coli</i> • N-terminal His-Patch thioredoxin fusion partner to improve protein translation efficiency and increase protein solubility

† For information on the parent vectors, check our web site at www.invitrogen.com

* Not commercially available.

Vector and recombined using the GATEWAY™ reaction into both the pcDNA-DEST47 and the pcDNA-DEST53 vectors. Figure 3 (page 10) clearly shows expression of the CAT/GFP fusion protein as evidenced by fluorescence of GFP.

better cloning and expression through GATEWAY™

GATEWAY™ offers the most comprehensive route to functional gene analysis and protein expression. To learn more about GATEWAY™, call the cloning and expression experts at Invitrogen or visit the online GATEWAY™ seminar at www.invitrogen.com.

Improve your gene analysis and protein expression. Call Invitrogen and order GATEWAY™ today.

Product	Quantity	Cat#	Price
pcDNA-DEST40	6 µg	12274-015	\$110
pcDNA-DEST53	6 µg	12288-015	\$140
pcDNA-DEST47	6 µg	12281-010	\$140
pEF-DEST51	6 µg	12285-011	\$110
pMT-DEST48	6 µg	12282-018	\$95
pYES-DEST52	6 µg	12286-019	\$95
pBAD-DEST49	6 µg	12283-016	\$110
GATEWAY™ LR CLONASE™ Enzyme Mix	20 rxns	11791-019	\$265
GATEWAY™ BP CLONASE™ Enzyme Mix	20 rxns	11789-013	\$265

new, simple licensing

GATEWAY™ now has simple licensing terms. For all internal research customers, you no longer need a license to use GATEWAY™ primers for PCR cloning, GATEWAY™ in high volume (>1,250 rxns./year), or advanced GATEWAY™ applications. Contact the Invitrogen Tech-Line or visit www.invitrogen.com for more information.

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